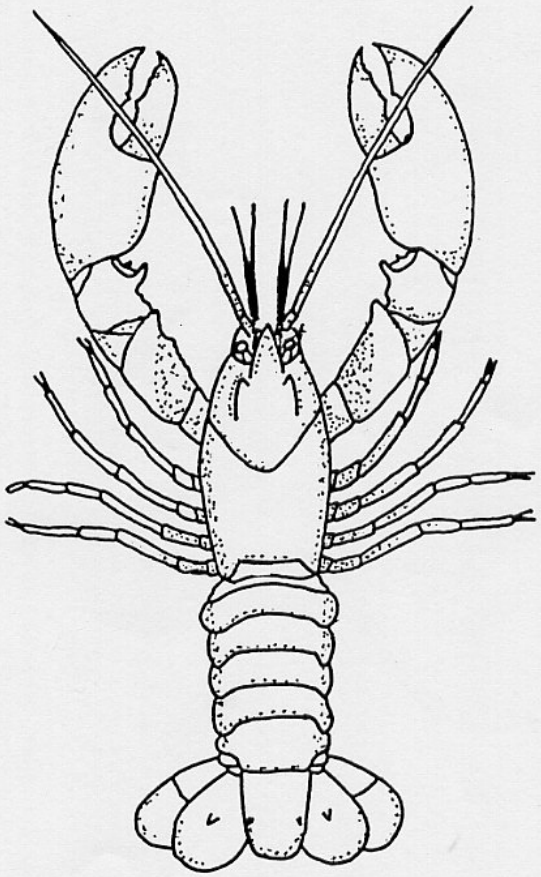
PARASITES AND ECTOCOMMENSALS OF YABBIES AND MARRON IN SOUTH AUSTRALIA



VETLAB REPORT

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YABBIE/MARRON HEALTH SURVEILLANCE PROJECT

FINAL REPORT

by

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Contents

INTRODUCTION

The commercial culture of freshwater crayfish is an expanding industry in many states of Australia. Since fish farm registration began in South Australia in July 1987, approximately 80 yabbie farms have been licensed in most regions of the state. Enterprises range from single dams on farms to large multi-dam ventures exceeding 10 hectares in size.

Native species of freshwater crayfish utilized for aquaculture throughout Australia include yabbies (Cherax destructor), marron (C. tenuimanus), redclaw or tropical blue crayfish (C. quadricarinatus) and gilgies (C. quinquecarinatus). The yabbie is the only species native to South Australia and their aquaculture has shown encouraging signs of developing into a viable industry. Nevertheless, producers have shown strong interest in importing other crayfish species from interstate for a variety of reasons. Some species grow to a larger size and are purported to have better growth characteristics and food conversion ratios. Other species are less aggressive and more suited to intensive culture. Considerable pressure has therefore been exerted on the South Australian Department of Fisheries to allow other crayfish species to be introduced into South Australia. However, any proposals for the interstate exchange of cultivated species must be considered carefully to determine any potential risks.

The introduction of any new non-native species into an area may have deleterious effects on local biotic communities through alterations in habitat, predation of native species or by competition for natural resources such as food, shelter, spawning sites, etc. The potential exists for escapees to form feral populations which may damage local ecosystems. In fact, a feral marron population has become established in a small area on Kangaroo Island but their impact on the local environment has not yet been assessed.

The introduction of a new species may also facilitate the dissemination of infectious diseases. Organisms in a balanced ecosystem have evolved and adapted with time to achieve enzootic stability whereby they survive pathogens; at least at the species level. The introduction of a new disease-causing organism can drastically affect populations that have no immune or defence mechanism for

that pathogen. In Europe, the freshwater crayfish aquaculture industry has been decimated by the introduction of the fungus Aphanomyces astaci on North American crayfish. If similar disasters are to be avoided, careful consideration must be given to requests to import new species and to establish appropriate quarantine and disease diagnostic services. To rationally assess the disease risk potential of introduced species, it is necessary to determine which organisms already exist in South Australia.

Preliminary studies performed on yabbies from South Australia have indicated that local populations may be relatively disease and parasite free. Two studies have failed to detect infections by the pathogenic microsporidian Thelohania or infestations by the presumed fungus Psorospermium and the epibiotic ciliate Cothurnia. In contrast, these organisms have been detected interstate on a range of other crayfish species. To avoid the introduction of these organisms into South Australia, it was proposed that imported species be subject to health certification testing for infections and infestations. The Australian Fish Health Reference Laboratory recently reviewed these proposals and made specific recommendations on health and quarantine measures required to prevent the importation of specified or notifiable diseases into the state. It was suggested that import restrictions were valid in the case of Thelohania infections because they are notorious pathogens of crayfish. However, import restrictions due to Cothurnia and Psorospermium infestations could not be easily justified due to their apparent lack of pathogenicity.

Nevertheless, no detailed studies have yet been performed in South Australia to determine which commensal and parasitic organisms occur in local crayfish populations. A comprehensive state-wide survey was therefore undertaken to examine farmed and wild crayfish populations for commensal and parasitic organisms.

MATERIALS AND METHODS

Origin of samples

A total of 2,850 freshwater crayfish were collected from 24 sites throughout South Australia (Figure 1). The sampling sites were located in 7 different geographic regions which correspond to natural drainage divisions. Where possible, farmed and wild crayfish populations were sampled from each region. A total of 2,548 yabbies (1,948 wild, 600 farmed) and 302 marron (150 feral, 152 farmed) were sampled in the period from October 1988 to April 1989.

All crayfish were captured live using baited wire-mesh nets and immediately transferred to shallow plastic tanks containing water from the sampling site to at least 10 cm depth. When transport to the laboratory occurred over a long period of time, the tanks were aerated using battery-operated aerators and the water was prevented from overheating by the addition of large blocks of ice.

Post-mortem examination

The crayfish were examined as soon as possible after their arrival at the laboratory. Each crayfish was killed by pithing with a fine scapel blade and the body was placed separately in a petri dish and covered with water from the tank. The sizes of the crayfish were determined by measuring the distance from the tip of the rostrum to the posterior end of the carapace along the mid-dorsal line (Figure 2). The sex of each crayfish was determined by examining the bases of the second and fourth pairs of walking legs for female and male genital openings respectively (Figure 2). The crayfish were then examined extensively for ectocommensal and endoparasitic organisms.

1. Ectocommensal organisms

   All external surfaces of the exoskeleton were scrutinized for attached organisms with particular attention paid to the telson and uropods (tail fans), the chelipeds (front claws) and the pleopods arising from the ventral abdominal surface (Figure 2). All four pairs of pleopods were excised and

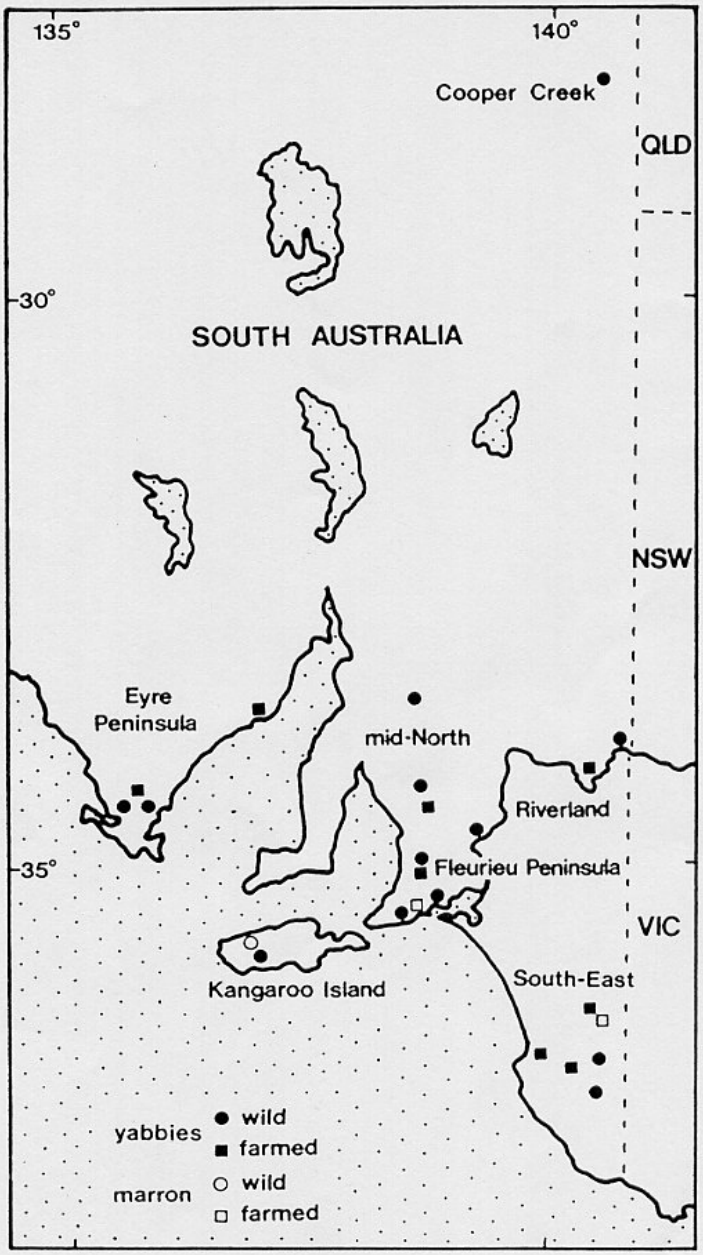

Figure 1. Geographic location of sampling sites in South Australia.

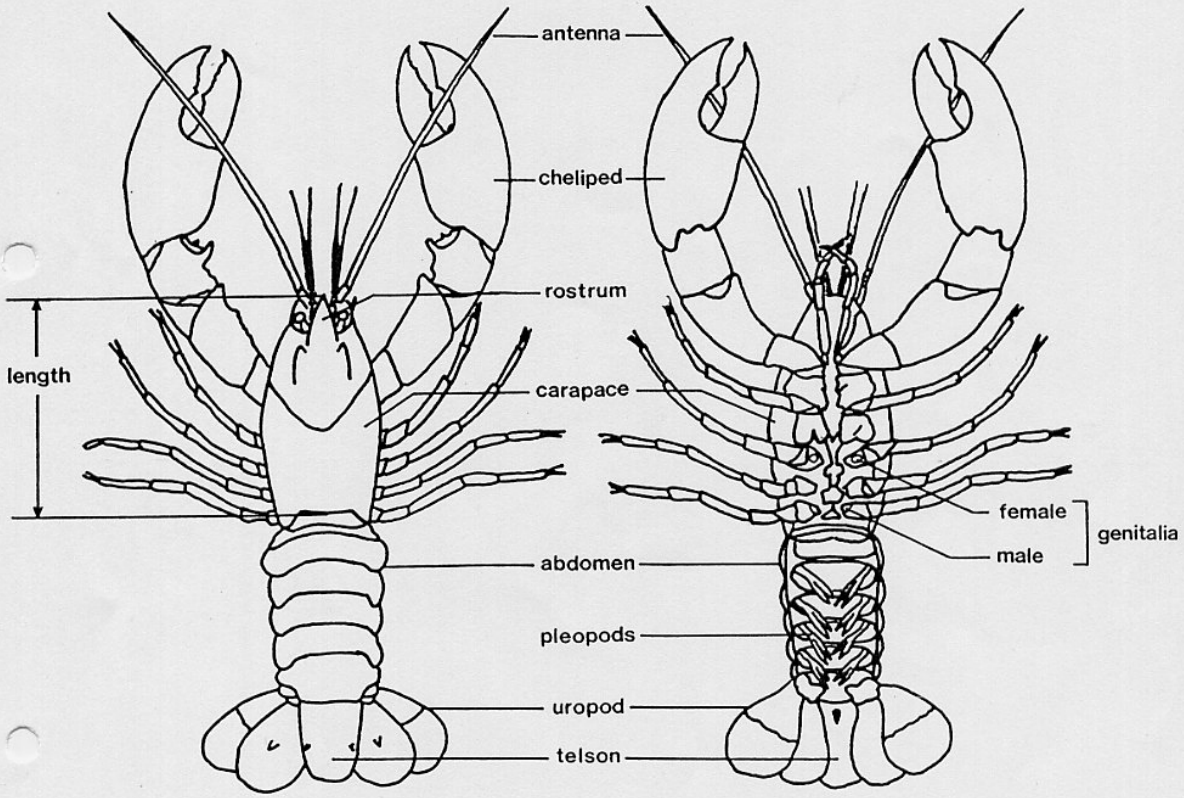

Figure 2. Morphological and anatomical features of freshwater crayfish.

placed in a separate petri dish and covered with water. The pleopods were examined under a dissecting microscope at 10-40x magnification. One pair of pleopods was then mounted in water on a slide and examined under a compound microscope at 100-400x magnification. Representative samples of ectocommensal organisms were collected and fixed for subsequent identification. Flatworms were fixed in Berland's fluid whereas protozoa were fixed in 10% formalin and Bouin's, Stieve's, Champy's and Parducz's fluids.

The carapace covering the gills was removed and the gill filaments were excised and placed in a separate petri dish filled with water. The gills filaments were examined under a dissecting microscope at 10-40x magnification. Four gill filaments were then mounted in water on a slide and examined under a compound microscope at 100-400x magnification. Nematodes were collected and fixed in Berland's fluid and protozoan organisms were fixed as described above.

2. Endoparasitic organisms

Each crayfish was transected at the junction of the thorax and abdomen. A cross-sectional slice of tail musculature (1 cm thick) was collected and fixed in 10% formalin. The exoskeleton was then removed from the remainder of the abdomen and the intestines were removed, mounted on a slide in water and examined under a compound light microscope at 40-400x magnification. Any helminths found within the gut were removed and fixed in Berland's fluid or 10% formalin.

Identification of organisms

All ectocommensal and endoparasitic organisms found in the crayfish were examined using standard protozoological and helminthological techniques and identified to at least the level of genus.

1. Protozoa

All ectocommensal ciliates were observed while alive and their key morphological features were noted (including size, colour, shape, movement, contractility and the presence and location of subcellular components such

as lorica, stalk, cytostome, somatic ciliation, contractile vacuole, operculum, tentacles, etc.). In the majority of cases, live observations were sufficient to identify organisms to generic level. However, histochemical and/or electron microscopic studies were required to identify organisms to species level.

Ciliates were silver impregnated to visualize kinety arrangements (patterns of ciliation) as well as to reveal nuclear structures. Live ciliates were air-dried onto albumenized slides and stained by the Klein-Foissner dry silver nitrate technique. Ciliates fixed in Bouin's and Stieve's fluids were enrobed onto slides in Mayer's albumen and stained with silver proteinate (protargol) whereas those fixed in Champy's fluid were enrobed onto slides in gelatin and stained by the Chatton-Lwoff wet silver nitrate technique. Kinety and nuclear arrangements were examined under a light microscope at 1,000x magnification using oil-immersion objectives. When necessary, topographical features were examined by scanning electron microscopy. Ciliates fixed in formalin and Parducz'z fluid were dehydrated in ethanol and then dessicated by air-drying or critical-point drying. Specimens were mounted on stubs, sputter-coated with gold and examined in a scanning electron microscope at 1,000-10,000x magnification.

Endoparasitic protozoa within yabbie tissues were examined by light and electron microscopy. Aliquots of the muscle samples which had been fixed in formalin were processed and embedded in paraffin wax using standard histological procedures. Tissue sections were cut at 5 um thickness and stained with haematoxylin and eosin, Giemsa and Ziehl-Neelsen stains using standard techniques. The sections were examined for microsporidian cysts under a light microscope at 100-1,000x magnification. Representative cysts were then cut from the paraffin blocks, de-paraffinized and processed into resin for transmission electron microscopy using standard techniques. Semi-thin survey sections were cut at 1 um thickness, stained with toluidine blue and examined under a light microscope at 400x magnification. Ultra-thin sections were then cut at 75 nm thickness and examined in a transmission electron microscope at 1,000-20,000x magnification to determine spore and cyst wall ultrastructure. The remainder of the formalin-fixed muscle samples were teased apart under a dissecting microscope and squash preparations were made which were examined under a light microscope at 400-1,000x magnification to determine sporoblast number and spore morphology.

2. Helminths

All ectocommensal platyhelminths (Temnocephala) were fixed in Berland's fluid, stained with Celestine blue, dehydrated in a graded series of ethanol solutions and mounted on slides in Canada balsam. Individual temnocephalans were examined under a light microscope at 100-1,000x magnification and identified by their morphological characteristics (including tentacle arrangement, organ location, presence of lamellae, pigmentation, etc).

Epizoic nematodes found in the gill cavities were collected and stored in Berland's fluid. Representative samples were mounted on slides in lactophenol and examined by light microscopy at 100-1,000x magnification. Adult nematodes were identified on the basis of their morphological characteristics (including size, oral structures, organ arrangement, etc).

Most helminths recovered from gut tissues were encysted larval stages which were dissected free of the host tissues prior to fixation. Nematode larvae were removed from cysts by fine forceps and fixed in Berland's fluid. Cestode larval stages were teased open with fine forceps to release the scolex and then fixed in formalin. Acanthocephalan cystacanths were relaxed by immersion in tap water to evaginate the proboscis and then fixed in 10% formalin. All fixed stages were mounted on slides and examined by light microscopy at 100-1,000x magnification. Where possible, taxa were identified to genus and species level according to their key morphological characteristics.

Lastly, muscle samples were examined for trematode metacercariae following routine histological preparation. Formalin-fixed muscles were embedded in paraffin wax and histological sections were cut at 5 um. thickness and stained with haematoxylin and eosin. The sections were examined under a light microscope at 40-400x magnification and the presence of metacercariae recorded. Fresh and formalin-fixed metacercariae were also teased open with fine forceps to release the contained immature trematodes which were identified by their morphological characteristics.

RESULTS AND DISCUSSION

A total of 17 invertebrate genera were detected living in association with freshwater crayfish either as commensal or parasitic organisms. These included unicellular protozoan organisms and multicellular metazoan flatworms, roundworms and thorny-headed worms; as follows:

Protista	- peritrichous ciliates	(6 genera)
(protozoa)	- suctorian ciliates	(2 genera)
	- microsporidia	(2 genera)
Platyhelminthes	- digeneans (flukes)	(1 genus)
(flatworms)	- temnocephalans	(2 genera)
	- cestodes (tapeworms)	(1 genus)
Nematoda	- free-living species	(1 genus)
(roundworms)	- parasitic species	(1 genus)
Acanthocephala		
(thorny-headed worms)	- parasitic species	(1 genus)
	TOTAL	17 genera

The percentage prevalence of infections or infestations by these organisms in wild and farmed yabbies and marron are summarized in Table 1 (page 12).

The percentage prevalence of infections or infestations in yabbies is presented for each geographic area in Table 2 (page 13).

TABLE 1. Percentage prevalence of ectocommensal and endoparasitic organisms in wild and farmed yabbies (*Cherax destructor*) and marron (*C. tenuimanus*)

	TOTAL	YABBIES	MARRON	WILD	FARMED
PROTISTA (protozoa)					
Ciliophora: Peritrichida					
Vaginicola spp.	19%	19%	16%	23%	8%
Cothurnia spp.	19%	20%	18%	26%	1%
Pyxicola spp.	15%	17%	1%	17%	10%
Lagenophrys spp.	49%	49%	49%	62%	13%
Epistylis spp.	61%	59%	77%	67%	45%
Vorticella spp.	37%	34%	55%	43%	19%
Ciliophora: Suctorida					
Acineta spp.	25%	27%	12%	30%	12%
Tokophrya sp.	0.1%	0.1%	–	0.1%	–
Microspora: Pleistophorida					
Thelohania sp.	5%	6%	–	7%	–
Pleistophora sp.	1%	1%	–	2%	–
PLATYHELMINTHES (flatworms)					
Trematoda: Digenea					
Microphallus minutus	15%	17%	–	20%	0.3%
Temnocephala: Temnocephalidea					
Craspedella spenceri	57%	63%	9%	57%	57%
Temnocephala dendyi	50%	50%	51%	55%	35%
Temnocephala sp. 1 (undescr.)	21%	24%	–	20%	27%
Temnocephala sp. 2 (undescr.)	0.3%	–	3%	–	1%
Cestoda: Cyclophyllidea					
Vampirolepis sp. (?*diminuta*)	0.3%	0.3%	–	0.4%	–
NEMATODA (roundworms)					
Chromadorea: Monhysterida					
Gammarinema sp.	62%	69%	–	72%	34%
Rhabditea: Spirurida					
spiruroid larvae	1.3%	1.4%	–	1.5%	0.7%
ACANTHOCEPHALA (thorny-headed worms)					
Palaeacanthocephala: Polymorphidae					
Polymorphus biziurae	0.3%	0.3%	–	0.3%	–
NUMBER CRAYFISH EXAMINED	2850	2548	302	2098	752

TABLE 2. Percentage prevalence of ectocommensal and endoparasitic organisms in yabbies (Cherax destructor) sampled from different geographic locations (SE = South-East; KI = Kangaroo Island; FP = Fleurieu Peninsula; EP = Eyre Peninsula; MN = Mid-North; RM = River Murray; OB = Outback)

	SE	KI	FP	EP	MN	RM	OB	TOT
PROTISTA (protozoa)								
Ciliophora: Peritrichida								
Vaginicola spp.	6%	18%	72%	2%	8%	21%	10%	19%
Cothurnia spp.	9%	2%	26%	0.2%	56%	13%	51%	20%
Pyxicola spp.	–	57%	69%	–	0.3%	11%	12%	17%
Lagenophrys spp.	39%	99%	98%	1%	–	75%	99%	49%
Epistylis spp.	42%	99%	73%	47%	76%	55%	54%	59%
Vorticella spp.	18%	35%	30%	78%	40%	43%	5%	34%
Ciliophora: Suctorida								
Acineta spp.	12%	41%	26%	26%	59%	13%	36%	22%
Tokophrya sp.	0.2%	–	–	–	–	–	–	0.1%
Microspora: Pleistophorida								
Thelohania sp.	0.4%	0.7%	0.3%	0.4%	38%	–	–	6%
Pleistophora sp.	4%	–	0.3%	–	5%	–	–	2%
PLATYHELMINTHES (flatworms)								
Trematoda: Digenea								
Microphallus minutus	27%	–	22%	6%	44%	2%	–	17%
Temnocephala: Temnocephalidea								
Craspedella spenceri	86%	–	73%	48%	52%	58%	100%	63%
Temnocephala dendyi	81%	85%	79%	13%	–	44%	85%	50%
Temnocephala sp. 1 (undescr.)	46%	1%	69%	14%	–	8%	1%	24%
Cestoda: Cyclophyllidea								
Vampirolepis sp. (?diminuta)	–	–	–	–	1%	1%	–	0.3%
NEMATODA (roundworms)								
Chromadorea: Monhysterida								
Gammarinema sp.	63%	99%	95%	20%	69%	81%	100%	69%
Rhabditea: Spirurida								
spiruroid larvae	1%	2%	2%	1%	–	2%	1%	1%
ACANTHOCEPHALA (thorny-headed worms)								
Palaeacanthocephala: Polymorphidae								
Polymorphus biziurae	0.4%	3%	–	–	–	0.2%	–	0.3%
NUMBER CRAYFISH EXAMINED	526	150	375	450	375	525	147	2548

* undescribed Temnocephala sp. 2 found only on 8 marron from SE

I. PROTOZOA (Kingdom Protista: Subkingdom Protozoa)

Two main types of protozoan organisms were detected in association with the freshwater crayfish; namely, ciliates (bearing hair-like cilia) and microsporidia (forming unicellular spores with polar filaments). All the ciliates were detected as ectocommensal or epibiotic organisms attached to the exoskeleton and/or gill filaments of the crayfish. In contrast, the microsporidia were detected as true endoparasites within the muscles of the crayfish.

A. CILIATES (Subkingdom Protozoa: Phylum Ciliophora)

A total of 8 ciliate genera were detected as ectocommensals on the crayfish; 6 genera of peritrichous ciliates (with ciliary spirals) and 2 genera of suctorian ciliates (with tentacles). Infestations by one or more ciliate genera were detected on 86% of the 2,850 crayfish examined (87% of 2,548 yabbies and 84% of 302 marron). Infestations were more prevalent on wild or feral crayfish populations (93%) than on farmed crayfish (68%).

Peritrichous ciliates (Class Oligohymenophora: Order Peritrichida)

All peritrichous ciliates are characterized by the possession of a left-hand spiral of cilia leading to the mouth (or cytostome). Six genera of peritrichous ciliates were detected on the crayfish and 18 species were identified following live observation, silver impregnation and morphometry. Ten species (belonging to 4 genera) were loricate ciliates where individual organisms (or zooids) were contained within shell-like houses (or loricae). The remaining 8 species (belonging to 2 genera) were not loricate but the zooids were mounted on long stalks. The morphological characteristics and prevalence and distribution of individual genera are presented on the following pages.

Vaginicola spp. (Order Peritrichida: Family Vaginicolidae)

Morphological characteristics (see Figures 3 and 4):

Individual zooids were elongate and trumpet-shaped when relaxed and extended. The zooids were highly contractile and withdrew rapidly into their loricae when disturbed. The loricae were clear and pseudochitinous in nature and measured approximately 110 um in length. The loricae were erect and upright and were attached at their bases directly to the substratum without an intervening stalk. Each lorica usually contained one or two zooids which were attached directly to the base of the lorica. The loricae did not have valves and the zooids did not have an operculum (lid or flap-like covering). Two species were identified; V. ampulla and an undescribed Vaginicola sp.

Prevalence and distribution:

Infestations by Vaginicola spp. were detected on 19% of the 2,850 crayfish examined. Infestations occurred predominantly on the gill filaments (90% of cases) whereas the remainder were found on pleopods. The intensities of infestation were usually not severe and organisms were found individually or in small clusters attached mainly to the basal branches of the gill filaments or pleopods. There was no significant difference between the prevalence of infestation on yabbies (19%) and marron (16%). More infestations were detected on wild yabbies (23%) than on farmed yabbies (8%) whereas all infestations on marron were found on farmed animals and none on feral animals. Infestations were detected on yabbies from all areas sampled throughout the state with the prevalence ranging from 2% on Eyre Peninsula to 72% on Fleurieu Peninsula.

Cothurnia sp. (Order Peritrichida: Family Vaginicolidae)

Morphological characteristics (see Figures 5 and 6):

Zooids were elongate and trumpet-shaped when relaxed but they contracted rapidly into clear pseudochitinous loricae when disturbed. The loricae were erect and upright measuring approximately 120 um in length. They were mounted on short outer stalks around 10 um in length which were attached to the substratum. The loricae were not valved and the zooids did not possess an operculum. The organisms detected in this study were very uniform in size, shape and structure and have all been assigned to a single unidentified Cothurnia sp.

Prevalence and distribution:

Infestations were detected on 19% of the crayfish; the majority being found on pleopods (97%) and the remainder on gill filaments. Loricae were mainly attached to the basal branches of the pleopods or gills and were usually found in small clusters but sometimes in large groups. The prevalence of infestations were similar on yabbies (20%) and marron (18%). Infestations were more prevalent on wild yabbies (26%) than on farmed yabbies (1%) whereas all infestations on marron were found on feral animals. Infestations were found in all areas throughout the state ranging in prevalence from 0.2% on Eyre Peninsula to 56% in the mid-North.

Pyxicola spp. (Order Peritrichida: Family Vaginicolidae)

Morphological characteristics (see Figure 7):

Zooids had elongate, trumpet-shaped bodies when relaxed but they contracted into pseudochitinous loricae when disturbed. The loricae were upright measuring approximately 120 um in length and they were mounted on short outer stalks around 10 um long which were attached to the substratum. The loricae did not contain valves but each zooid possessed a discoid operculum situated beneath the peristomal border. The operculum served to seal the lorica aperture when the animal contracted. Two species were identified; *P. carteri* and *P. pusilla*.

Prevalence and distribution:

Infestations were detected on 15% of the crayfish and all organisms were found only on pleopods. The levels of infestation were generally low and most organisms were found as solitary individuals attached to the basal branches of the pleopods. More infestations were found on yabbies (17%) than on marron (1%). More infestations were detected on wild yabbies (17%) than on farmed yabbies (10%) whereas only feral marron were found to be infested. Infestations were notably absent in the South-East and on Eyre Peninsula whereas their prevalence throughout the rest of the state ranged from 0.3% in the mid-North to 69% on Fleurieu Peninsula.

Lagenophrys spp. (Order Peritrichida: Family Lagenophryidae)

Morphological characteristics (see Figures 8, 9 and 10):

The zooids had rounded bodies which were enclosed in discoid pseudochitinous loricae lying flat on the substratum. The loricae were round and dorso-ventrally flattened measuring approximately 80 um in diameter. The peristomal region of the zooid extended out of an aperture towards the apex of the lorica. The aperture was surrounded by a neck-like collar region which was simple or complicated in structure and sometimes contained projecting spines. The loricae did not have valves and the zooids did not have an operculum. Five species were identified by comparative morphological studies; *L. spinosa*, *L. lingulata*, *L. darwini*, *L. communis* and *L. willisi*.

Prevalence and distribution:

Infestations by *Lagenophrys* spp. were detected on 49% of the crayfish; the majority involving gill filaments (99%) although organisms were frequently detected on pleopods (39%). Infestations were usually quite heavy and the gill filaments were sometimes covered with ciliates. The prevalence of infestations was similar on yabbies and marron (both 49%). Infestations were more prevalent on wild yabbies (62%) than on farmed yabbies (13%) whereas all infested marron were feral animals. No infestations were found on yabbies from the mid-North and they were low in prevalence on Eyre Peninsula (1%). Infestations were more prevalent throughout the rest of the state ranging in prevalence from 39% in the South-East to 99% on Kangaroo Island and in the Outback.

Epistylis spp. (Order Peritrichida: Family Epistylididae)

Morphological characteristics (see Figures 11, 12 and 13):

The zooids had inverted bell-shaped bodies which measured approximately 100 um in length when relaxed. The zooids contracted into rounded bud-shapes when disturbed. The peristomal lips of the contracted zooids were forced into prominent nipple-like shapes and their posterior pellicles contained distinct folds or ridges. The zooids were not encased within loricae but were mounted on long, branched, non-contractile stalks. The organisms were colonial and formed small to large branching colonies which were often attached to the substratum by a single stalk. Two species were identified by silver impregnation; *E. variabilis* and one undescribed *Epistylis* species.

Prevalence and distribution:

Colonies of *Epistylis* spp. were detected on 61% of the crayfish examined; the majority (99%) occurring on the pleopods and the remainder attached to gill filaments. Although the colonies generally contained many individuals, they were frequently patchy in their distribution over individual crayfish. Nevertheless, some crayfish were found literally covered with organisms. Infestations were more prevalent on marron (77%) than on yabbies (59%) and more infestations were detected on wild crayfish (67%) than on farmed animals (45%). Infestations were found in all areas ranging in prevalence from 42% in the South-East to 99% on Kangaroo Island.

Vorticella spp. (Order Peritrichida: Family Vorticellidae)

Morphological characteristics (see Figures 14 and 15):

The zooids had inverted bell-shaped bodies measuring approximately 80 um in length when relaxed. The zooids contracted when disturbed becoming small and rounded but lacking distinctive features apart from the inverted peristomal region which assumed a puckered appearance. The zooids were not enclosed in loricae but were mounted on long, non-branched stalks. Each stalk contained a single sinusoidal myoneme (contractile fibril). The stalks were variable in length and contracted rapidly in a spiral fashion thereby giving the impression that the organisms were coiling when disturbed. The organisms were solitary in nature and were found as individual organisms although some were gregarious and formed clusters of many individuals whose stalks were intertwined or attached to each other. Six species were identified following silver impregnation; V. jaerae, V. convallaria, V. calciformis, V. flexulosa and 2 undescribed Vorticella species.

Prevalence and distribution:

Infestations were detected on 37% of the crayfish examined; the majority being associated with pleopods (99%) and the remainder with gill filaments. Infestations by solitary individuals and clusters of organisms were usually few in number and patchy in distribution. The prevalence of infestations was greater on marron (55%) than on yabbies (34%) and also greater on crayfish from wild populations (43%) than on those from farmed populations (19%). Infestations were present on crayfish from all areas examined and the prevalence ranged from 5% in the Outback to 78% on Eyre Peninsula.

Significance of infestations by peritrichous ciliates

All peritrichous ciliates were found as ectocommensal or fouling organisms and infestations were not associated with any clinical or subclinical disease. However, previous studies have suggested that heavy infestations may be responsible for some losses through anoxia and/or impaired gill function. In this study, only infestations by Lagenophrys spp. were sometimes found to be so dense that the gill filaments were covered with organisms. It is not known whether such infestations impaired gill function.

Crayfish moult frequently as they grow therefore ectocommensal organisms are shed with the old exoskeleton. However, peritrichous ciliates can detach from unfavourable substrates and form telotroch stages which actively seek more favourable locations. Re-infestation can occur quite rapidly when telotroch stages settle on new substrates and become attached. It is not known whether heavy infestations by fouling organisms affect crayfish growth characteristics or moulting frequency.

Peritrichous ciliates are generally filter-feeding bactivores and occur in large numbers in waters which contain dense bacterial populations due to high nutrient loads. Heavy infestations are therefore thought to be indicative of high organic pollution and several correlations have been found between peritrich loads and various water quality parameters. Sound pond management practices such as avoiding overfeeding and regularly changing pond water have been found to alleviate or eliminate problems with heavy infestations. Severe infestations usually subside as water quality improves. Some measure of control has also been reported by treatment with weak formalin or acetic acid baths.

Due to the widespread distribution and abundance of these organisms and their apparent lack of pathogenicity, they are not considered to pose a real threat to the aquaculture industry. It has even been suggested that they be incorporated into biological monitoring programmes as indicators of poor water quality. Any recommendations to restrict imports of crayfish due to the presence of these organisms could not be easily justified.

Suctorian ciliates (Class Kinetofragminophora: Order Suctorida)

All suctorian ciliates are characterized by the possession of ingestatory suctorial tentacles. Two genera of suctorian ciliates were detected on the freshwater crayfish and 3 species were identified following live observation and silver impregnation.

Acineta spp. (Order Suctorida: Family Acinetidae)

Morphological characteristics (see Figures 16 and 17):

The ciliates had inverted conical shaped bodies measuring approximately 120 um in length. The bodies were flattened and housed within cup-like loricae which were close-fitting and difficult to observe in live animals. The loricae were mounted on stalks of variable lengths which were attached to the substratum. The body of each ciliate contained 2 distinct fascicles of tentacles which were capitate. Two species were identified; A. tuberosa and A. fluviatilis.

Prevalence and distribution:

Infestations were found on 25% of the crayfish examined; the majority occurring on pleopods (99%) and only several infestations being found on gill filaments. The organisms were usually found as individuals attached to the basal branches of the pleopods and infestations were not severe. Infestations were more prevalent on yabbies (27%) than on marron (12%). More infestations were found on wild yabbies (30%) than on farmed yabbies (12%) whereas more were found on farmed marron (20%) than on feral marron (3%). Infestations were detected in all areas of the state ranging in prevalence from 12% in the South-East to 59% in the mid-North.

Tokophrya sp. (Order Suctorida: Family Dendrosomatidae)

Morphological characteristics (see Figure 18):

The ciliates had inverted pyriform shaped bodies measuring approximately 140 um in length. The bodies were rounded in cross-section and not housed in loricae but were mounted on flexible stalks of variable lengths. The tentacles were capitate and arranged in 2-4 fascicles. Only one species was identified; T. cyclopum.

Prevalence and distribution:

Mild Tokophrya infestations were detected only on the pleopods of one wild yabbie which originated from the South-East.

Significance of infestations by suctoria

The suctorian ciliates were not apparently associated with any host disease or mortality. Previous studies have indicated that heavy infestations on marine penaeid prawns may interfere with feeding, locomotion or respiration and may even cause mortality when gills are severely fouled. However, the infestations found on the freshwater crayfish were generally light in intensity and were usually associated with external surfaces and not gill filaments. Most suctoria are sedentary as adults and occur as ectocommensals on a variety of substrates. Those found on the exoskeletons of crayfish would therefore be shed from the host during moulting. However, re-infestation occurs when adult suctoria form motile ciliated larval stages which seek out more favourable locations. It is not known whether suctoria affect crayfish growth or moulting. The majority of suctoria feed on other organisms (particularly other protozoa) which are trapped by the tentacles. Heavy infestations require plentiful prey organisms which usually indicates poor water quality with high organic content. Infestations can be alleviated by eliminating excessive feeding and changing pond water.

The levels of infestation found in this study were not considered to be detrimental to crayfish aquaculture. Organisms were relatively widespread throughout the state and there is no solid evidence to support recommendations to restrict imports of infested crayfish.

B. MICROSPORIDIA (Subkingdom Protozoa: Phylum Microspora)

The microsporidia are small, spore-forming, intracellular parasites which form unicellular spores containing coiled polar filaments. Mature spores are generally aggregated together in colonies or cysts within the host tissues. Infections by microsporidian cysts were found in the muscles of 5.9% of the 2,850 crayfish examined. All infections were detected in yabbies sampled from wild populations and none were found in farmed yabbies.

Two distinct types of cysts were detected in the muscles of the yabbies; thin elongate cysts and large globular cysts. Both types of cysts contained mature spores which were uninucleate; thereby placing them in the same order (Pleistophoridida). However, the sporophorous vesicles in the cysts contained different numbers of sporoblasts and developing spores; thereby placing them in separate families and genera. The morphological characteristics and prevalence and distribution of the microsporidian infections are presented below.

Thelohania sp. (Order Pleistophoridida: Family Thelohaniidae)

Morphological characteristics (see Figures 19, 20 and 21):

All microsporidian developmental stages were contained within thin elongate cysts (xenomas) in the host musculature. The parasites were characterized by the occurence of octosporoblastic sporogony (sporonts each producing 8 sporoblasts). The sporoblasts were enclosed in sac-like structures known as sporophorous vesicles (formerly called pansporoblasts). Each vesicle contained 8 sporoblasts which matured without further division into uninucleate spores. The mature spores were monomorphic (uniform in size and shape); being pyriform and measuring approximately 3 um in length by 2 um in width. The spores were very refractile in transmitted light and were phase-bright.

Heavily-infected muscle fibres appeared opaque and mottly white in colour instead of clear and translucent (thereby justifying the use of the terms 'cotton-tail' or 'milky-tail' for such conditions). Spore morphology and sporoblast number was best determined by the microscopic examination of squash preparations of infected musculature whereas cyst and cyst wall architecture was best observed in histological sections. The elongate cysts measured up to 2 mm in length and ranged from 20-80 um in width. The cyst walls were smooth and thin and did not contain invaginations or septae. The spores were lightly basophilic whereas sporonts and sporoblasts were eosinophilic. All structures stained much better with Giemsa or Ziehl-Neelsen stains than with haematoxylin and eosin. Details of spore morphology could only be determined by electron microscopic examination. The spore wall was composed of three distinct layers and the coiled polar filament had 6-7 windings. These microsporidia were very uniform in morphology and ultrastructure and are believed to belong to a single undescribed Thelohania species.

Prevalence and distribution:

Infections were detected in 5.8% of the 2,548 yabbies examined (i.e. in 148 yabbies) and all infected yabbies were sampled from wild populations. No infections were detected in marron. The intensities of infection in individual yabbies were very high and involved numerous muscle fibres and bundles. In several instances, parasitized muscle fibres outnumbered non-parasitized muscle fibres. Many infections were also accompanied by myodegeneration and focal infiltrates of inflammatory cells.

No infections were detected in yabbies sampled from the River Murray or the Outback. The prevalence of infections was highest in the mid-North (38%) where all infected yabbies were caught in 2 river systems (127 in the Broughton River and 15 in the River Light). Infections were very low in prevalence throughout the remainder of the state (0.3-0.7%) and only involved several individual animals (1 from Kangaroo Island, 1 from Fleurieu Peninsula, 2 from the South-East and 2 from Eyre Peninsula).

Pleistophora sp. (Order Pleistophoridida: Family Pleistophoridae)

Morphological characteristics (see Figures 22, 23, 24, 25 and 26):

All parasitic developmental stages were contained within large ovoid intramuscular cysts bounded by thickened walls. The parasites were characterized by the occurence of polysporoblastic plasmodial sporogony (successive fragmentation of multinucleate sporont or plasmodium producing irregular numbers of sporoblasts). The sporoblasts were contained within sporophorous vesicles which had thick envelopes and each vesicle contained large numbers of sporoblasts which matured into uninucleate spores. Dimorphic sporogony occurred whereby two different types of spores were formed. The majority of vesicles contained large ovoid spores measuring approximately 2 um in length by 1.5 um in width whereas the remainder contained smaller rounded microspores measuring approximately 1 um in diameter. Only the larger spores were refractile in transmitted light whereas both types were moderately phase-bright.

The intact cysts appeared as dark rounded bodies within the normally translucent host muscles. Cysts were easily dissected from host musculature and spore morphology and arrangement was readily determined by the microscopic examination of squash prepartions. Histological examination revealed large rounded cysts measuring from 300-700 um in diameter which were surrounded by distinct walls approximately 2 um thick. Spores stained more intensely with Giemsa and Ziehl-Neelsen stains than with haematoxylin and eosin. Ultrastructural examination revealed the spore walls to consist of three layers and the coiled polar filaments to have 8-9 windings. The majority of cysts detected were similar in morphology and are thought to belong to the same undescribed Pleistophora species.

Prevalence and distribution:

Infections were detected in 1.5% of the 2,548 yabbies (i.e. in 39 animals) and all infected yabbies were sampled from wild populations. No infections were found in marron. All cysts were detected in the abdominal muscles of the yabbies and the intensities of infection were generally low with only several cysts being detected in each yabbie. Infections were only detected

in yabbies sampled from 3 geographic locations where the prevalence of infection was low; 0.3% on Fleurieu Peninsula (1 yabbie), 4% in the South-East (19 yabbies) and 5% in the mid-North (19 yabbies).

Hyperparasitism (see Figures 29 and 30):

In addition to the characteristic microsporidial infections described above, several infections were also found in association with trematode metacercarial stages encysted within yabbie muscles. Large rounded cysts packed with masses of mature spores were also found to contain the remnants of 'suckers'; structures typical of immature trematodes (or metacercariae). The cysts were similar in size to metacercariae (210-250 um in diameter) but they did not contain any other recognizable internal or external structures due to their apparent degenerative state. Nevertheless, the presence of the 'suckers' was highly suggestive that they were in fact metacercariae which were infected with microsporidia; thereby representing another instance of hyperparasitism (i.e. a parasitic infection of another parasite).

The morphological characteristics of the microsporidian spores and their vesicle-like compartments were similar to those previously observed in Pleistophora cysts. Infections of metacercariae with microsporidia were only detected in 4 yabbies which all originated from a wild population in the mid-North of the state (from the Broughton River). Concomitant infections by metacercariae of the trematode Microphallus minutus and cysts of the microsporidian Pleistophora were found in the muscles of the same 4 yabbies. The significance of the microsporidial infections detected within the trematode metacercariae is unknown although they may simply be opportunistic in nature.

Significance of infections by microsporidia

Two genera of microsporidia were detected as endoparasites in the muscles of yabbies. Infections by Pleistophora were low in prevalence and intensity whereas those by Thelohania were more prevalent and usually very heavy in intensity. Infected muscles were frequently discoloured and degenerate making them unsuitable for marketing. Thelohania spp. are regarded as notorious pathogens of crustacea and serious economic losses have been attributed to animal mortalities and loss of acceptable product. The potential therefore exists for microsporidian infections to seriously affect freshwater crayfish aquaculture.

It is presumed that yabbies become infected following the ingestion of mature spores which extrude their polar filaments and inject the infective sporoplasm into a host cell. The sporoplasm firstly proliferates by merogony and sporoblasts are then formed by sporogony within sporophorous vesicles. The sporoblasts develop into mature spores which are contained within intramuscular cysts until their release by lesion rupture or death of the host. The actual mode of transmission between hosts is not known but it may be direct involving cannibilism or indirect involving another host animal. The removal of sick and dead yabbies from ponds and the elimination of predatory or scavenging animals may reduce the risks of transmission. At present, there is no known treatment for microsporidiosis in crustacea although trials with several new drugs have shown limited efficacy in disrupting early developmental stages of microsporidia in insects and fish.

The majority of microsporidian infections were detected in yabbies from 2 river systems in the mid-North of the state (Broughton River and River Light). It is therefore recommended that farms not be stocked with crayfish from these rivers due to the prevalence of infections. Small numbers of infected yabbies were also found elsewhere in the state and it is suggested that crayfish from these areas be monitored in the future for increases in the incidence of infections. Previous studies have detected Thelohania infections in crayfish from other interstate areas therefore it is imperative that imported crayfish be subject to disease-free certification to avoid the introduction of further infections.

II. PLATYHELMINTHS (Kingdom Animalia: Phylum Platyhelminthes)

Three types of platyhelminths (flatworms) were detected in association with the crayfish; namely, trematodes or flukes (with alimentary canals), temnocephalans (free-living or ectocommensals) and cestodes or tapeworms (without alimentary canals). The trematodes and cestodes were found as endoparasites within host tissues whereas the temnocephalans were found as ectocommensals on the crayfish.

A. DIGENETIC TREMATODES (Class Trematoda: Order Digenea)

Developmental stages of one trematode species were detected within the muscles of 15% of the 2,850 crayfish examined. The stages detected were encysted final larval stages (or metacercariae) which are indicative of an indirect (or digenetic) life cycle requiring a definitive host and at least one intermediate host.

Microphallus sp. (Order Digenea: Family Microphallidae)

Morphological characteristics (see Figures 27, 28, 29 and 30):

Encysted metacercarial stages were detected in the abdominal and gut muscles of the crayfish. The cysts were translucent and almost colourless in appearance. They were spherical in shape measuring from 210-250 um in diameter and were bounded by double-layered rigid walls ranging from 10-25 um in thickness. The cyst walls were eosinophilic, PAS positive and stained well with Giemsa and Ziehl-Neelsen stains. The cysts contained single immature metacercariae which had two suckers and well developed alimentary canals and genitalia. The morphological characteristics of the immature flukes were similar to those of the species *M. minutus* which is parasitic in water rats.

Prevalence and distribution:

Infections were detected in 430 (17%) of the 2,548 yabbies; the majority originating from wild populations and only 2 from farmed populations. No

infections were detected in marron. The intensities of infection were generally moderate except in a few individuals where numerous metacercariae were detected. No infections were detected in yabbies from Kangaroo Island or the Outback whereas the prevalence of infections elsewhere in the state ranged from 2% in the River Murray to 44% in the mid-North.

Significance of trematode infections

The trematode metacercarial stages detected in the host muscles were not apparently associated with any clinical disease. Nevertheless, their presence in large numbers caused an unsightly appearance of the musculature resulting in its decreased commercial value. The metacercariae may also account for a gritty sensation experienced when infected muscles are eaten.

Infections in crustacea by trematode metacercariae form following the penetration or ingestion of cercarial stages released by molluscs (usually snails). The metacercariae are the infective final larval stages which must be ingested by a definitive host (presumably water rats) before maturation into adult flukes can occur. Infections could therefore be controlled by reducing or eliminating mollusc populations from ponds and inlet waters and by restricting the access of predatory hosts to infected yabbies. Molluscs may be eliminated through the use of molluscicides, draining and drying ponds or using quicklime or chloride of lime. At present, no treatment is available for infections by metacercarial stages.

Because infections by metacercariae were only prevalent in some areas of the state, attempts should be made to reduce the risk of their distribution to other areas or their introduction into aquaculture facilities. Infected wild stock should not be used to establish cultures unless some attempts are made to free ponds of molluscs and make them secure against predators. Where this is not possible, wild stock should be examined for infections before their introduction into culture ponds. It is not known whether infections occur in crayfish from interstate areas but imported crayfish should be examined for infections to avoid their introduction into new areas.

B. TEMNOCEPHALA (Class Temnocephala: Order Temnocephalidea)

Temnocephalidean platyhelminths were detected as ectocommensal organisms on the external surfaces of the crayfish. Adults were detected browsing over the exoskeleton and gills whereas their eggs were found firmly attached to the exoskeleton (particularly on ventral abdominal surfaces) and also on the gills. The adults possessed anterior tentacles and a posterior ventral sucker which was used for attachment.

Four species belonging to 2 genera were identified according to the morphological characteristics of the adults. Infestations by one or more species were detected on 84% of the crayfish examined. Infestations were slightly more prevalent on yabbies (84%) than on marron (79%) but were equally prevalent on crayfish from wild populations (84%) and farmed populations (83%). The morphological characteristics and prevalence and distribution of the 4 species are described on the following pages.

Craspedella spenceri (Order Temnocephalidea: Family Temnocephalidae)

Morphological characteristics (see Figure 31):

Adults were dorsoventrally flattened and elongate in shape measuring up to 1.5 mm in length. The adults were moderately motile, translucent and pale white in appearance and possessed 5 slender tentacles at the anterior end and a single posterior ventral sucker. The body was highly contractile and its surface was ruffled in appearance. The adults contained 3 prominent frills or transverse lamellae in the mid-body region and 5 radial lamellae towards the posterior end. Two dark eyespots were evident at the anterior end but there were no other indications of pigmentation.

Prevalence and distribution:

Infestations were detected on 57% of the crayfish examined and they were more prevalent on yabbies (63%) than on marron (9%). Infestations were similar in prevalence on crayfish from wild and farmed populations (both 57%). Infestations were more prevalent in the gill cavities than on the exoskeleton. No infestations were detected on crayfish from Kangaroo Island whereas they were very prevalent throughout the rest of the state ranging in prevalence from 48% on Eyre Peninsula to 100% in the Outback.

Temnocephala dendyi (Order Temnocephalidea: Family Temnocephalidae)

Morphological characteristcis (see Figure 32):

The adults were elongate and slender in shape when extended measuring up to 3 mm in length. Although the adults were very contractile, their movement was usually slow and deliberate. The adults possessed 5 plump anterior tentacles and a single posterior ventral sucker. The body was opaque white in appearance and its surface was smooth without ruffles or lamellae. Two eyespots were prominent at the anterior end but there were no other signs of pigmentation.

Prevalence and distribution:

Infestations were detected on 50% of the crayfish examined. The prevalence of infestations was similar on yabbies (50%) and on marron (51%) but more infestations were found on crayfish from wild populations (55%) than from farmed populations (35%). Most organisms were found within crevices formed by joints in the exoskeleton. No infestations were found in the mid-North whereas they were frequently detected in all other areas ranging in prevalence from 13% on Eyre Peninsula to 85% on Kangaroo Island.

Temnocephala sp. (Order Temnocephalidea: Family Temnocephalidae)

Morphological characteristics (see Figures 33 and 34):

The adults were elongate and plump in shape measuring up to 2 mm in length. They were highly motile and contained 5 anterior tentacles and a single ventral sucker. The body was translucent with a smooth surface but was characterized by a clearly-visible anastomosing network of darkly pigmented fibres (neural elements) throughout the body. Paired sensory structures were present on either side of the posterior sucker. These structures were similar to those previously described in a non-pigmented species from Western Australia. However, it is thought the pigmented species has not previously been described.

Prevalence and distribution:

Infestations were found on 21% of the crayfish examined; all being detected on yabbies only. Infestations were slightly more prevalent on farmed yabbies (27%) than on wild yabbies (20%). The majority of organisms were found on ventral abdominal surfaces. No infestations were found on yabbies from the mid-North whereas their prevalence varied considerably elsewhere ranging from 1% on Kangaroo Island and in the Outback to 69% on Fleurieu Peninsula.

Temnocephala sp. (Order Temnocephalidea: Family Temnocephalidae)

Morphological characteristics:

The adults were elongate and squat in appearance measuring up to 2 mm in length. They were highly motile and possessed 5 slender tentacles and a ventral sucker. The body was translucent in appearance and possessed several slight ruffles or lamellae but did not contain pigmented fibres. The morphological characteristics of this species were intermediary between *Craspedella* and *Temnocephala* and have not previously been described.

Prevalence and distribution:

Infestations were only detected on 8 marron which all originated from a single farm in the South-East of the state. Most organisms were found on ventral abdominal surfaces.

Significance of temnocephalan infestations

The temnocephalans were detected as ectocommensals on both yabbies and marron and were not directly associated with any clinical disease. However, previous studies have indicated that heavy infestations in the gill cavities can affect respiratory efficiency, cause hypoxia and even result in death. Although the infestations detected in this study were generally mild (involving from 1-100 adults), the presence of numerous temnocephalan eggs on the external surfaces of some crayfish made them unsightly and may lead to consumer resistance. The eggs are strongly adherent and remain after steaming or boiling.

Adult temnocephalans browse on the surfaces of the host eating planktonic organisms and small invertebrates. They are not considered to utilize the same food sources as their hosts. Temnocephalans have a direct life cycle whereby the hermaphroditic adults attach eggs to substrates and the eggs subsequently hatch releasing immature juvenile forms. Infestations are difficult to eradicate due to the resistant nature of the eggs. Formalin treatment appears effective in eradicating adults but does not work against eggs. Repeat treatments are needed after the eggs hatch but before the juveniles mature and lay eggs. Alternatively, ponds can be drained and dried and then restocked with non-infested crayfish.

The high prevalence and wide distribution of infestations on wild and farmed crayfish throughout the state would appear to preclude any restrictions placed on the movement of stock. The certification of imported species as free from infestations would also be difficult to justify but some effort should be made to prevent their introduction into new areas. A number of temnocephalan species described from various regions in other states were not encountered in this survey and it seems prudent to prevent their introduction into South Australia. Infested stock should either be avoided or treated prior to their release into ponds.

C. CESTODES (Class Cestoda: Order Cyclophyllidea)

Developmental stages of one cestode species were detected in 0.3% of the crayfish examined. The parasites were detected as encysted larval stages (metacestodes) indicating they have an indirect life cycle requiring a definitive host for the development of the adult tapeworm.

Vampirolepis sp. (Order Cyclophyllidea: Family Hymenolepididae)

Morphological characteristics (see Figures 35 and 36):

Ovoid cream-coloured cysts measuring approximately 1 mm in diameter were found embedded in the intestinal mucosa of the crayfish. The cysts were classified as cysticercoid stages because they consisted of single vesicles bounded by thick walls and each contained a single non-invaginated tapeworm head (scolex). Cysts were teased open to release the scolex which was found to possess 4 unarmed suckers, a retractable rostellum bearing a single circle of hooks and a prominent cercomer (or tail). These morphological features were similar to those of the genus Vampirolepis and the species was tentatively identified as V. diminuta. However, attempts to experimentally infect laboratory rats were unsuccessful.

Prevalence and distribution:

Infections were only detected in 8 yabbies which all originated from wild populations; 4 from the mid-North and 4 from the River Murray. Most infections involved single cysts only.

Significance of cestode infections

Infections by cestodes were detected as encysted cysticercoid stages in the tissues of the yabbies which served as intermediate hosts for the parasites. The cysticercoid stages must be ingested by another host species which serves as the definitive host for the adult tapeworm. The definitive host must therefore be a predator or scavenger on yabbies. Although the actual definitive host is not known, the cestode species was similar to others previously described in rodents. Infections in culture ponds may therefore by avoided by preventing the access of wild animals (particularly rodents). The water rat Hydromys chrysogaster commonly preys on yabbies and harbours infections by V. diminuta which has a metacestode stage identical to that encountered in the yabbies.

The cysticercoid stages were not detected in association with any clinical disease nor any overt pathological changes in the gut wall. They are therefore not regarded as significant pathogens of crayfish. Infections were very low in prevalence and were only detected in wild populations from two geographic locations. It therefore appears that there are no valid reasons to justify any restrictions placed on the movement of stock due to infections by these cestodes.

III. NEMATODES (Kingdom Animalia: Phylum Nematoda)

Two genera of nematodes (roundworms) were found living in association with the crayfish. All worms were typically unsegmented, cylindrical and elongate in shape and possessed complete digestive tracts including mouth, oesophagus, intestine and anus. Free-living nematodes (with sensory setae or tactile receptors) were detected in the gill cavities whereas parasitic nematodes (without setae) were found in the gut.

A. Free-living nematodes (Class Chromadorea: Order Monhysterida)

The roundworms detected within the gill chambers were all adult stages belonging to a single free-living nematode species. Numerous species of free-living nematodes have been described around the world in a variety of aquatic environments sometimes living in association with various invertebrate and vertebrate hosts. Because these species do not have a true symbiotic or commensal relationship with the host, they are often refered to as epizoic species.

Gammarinema sp. (Order Monhysterida: Family Monhysteridae)

Morphological characteristics (see Figures 37, 38 and 39):

The adult nematodes were cylindrical and elongate in shape measuring from 1.6-2.0 mm in length. The worms were highly motile and translucent in appearance although the digestive tract was sometimes pale brown in colour. The worms were lying free within the gill chambers and were occasionally entangled in the gill filaments. The mouths of the worms opened terminally and were surrounded by 3 lips each bearing 2 sensory papillae. The oesophagus was distinctly divided into 2 parts and the anus opened subterminally. The external cuticle contained setae appearing as thin elongate projections or hairs at regular intervals along the body. The worms were either female with genitalia opening into a vulva or male with genitalia opening into the distal intestine and a pair of copulatory spicules associated with the cloaca. The worms are thought to belong to a single unidentifed *Gammarinema* species.

Prevalence and distribution:

Infestations were detected in 62% of the crayfish examined. All infestations were detected in yabbies (69%) and none in marron. Infestations were more prevalent in wild yabbies (72%) than in farmed yabbies (34%). The intensities of infestation varied considerably involving from 1-100 individual worms. Infestations were found in yabbies sampled throughout the state ranging in prevalence from 20% on Eyre Peninsula to 100% in the Outback.

Significance of infestations by free-living nematodes

The nematode infestations detected in the gill cavities were not associated with any clinical signs of disease or any pathological condition. Previous studies have reported free-living nematodes infesting the gills of yabbies but their effects on the host are not known. The nematodes presumably have a direct life cycle where all developmental stages are free-living. Infestations were prevalent throughout the state but they were only found on yabbies (both wild and farmed). It is not known, however, whether these nematodes were host specific for yabbies only. Nonetheless, infestations by these epizoic nematodes are not thought to be of concern to the aquaculture industry due to their apparent lack of pathogenicity.

B. Parasitic nematodes (Class Rhabditea: Order Spirurida)

The roundworms detected in the gut walls of the crayfish were larval stages of a single parasitic nematode species. Because only larvae and no adults were detected, their identification even to genus level was not possible. Nevertheless, the larvae were found to have various morphological features common to spiruroid nematodes (oesophagus with short anterior muscular region and long posterior glandular region).

Spiruroid nematodes (Order Spirurida: Family undetermined)

Morphological characteristics (see Figures 40, 41 and 42):

Small transparent nodules approximately 1-1.5 mm in diameter were detected in the intestinal walls of the crayfish. The nodules were found to contain single nematode larvae which were cylindrical and elongate in shape ranging from 2.4-2.6 mm in length. The mouths of the larvae contained 2 lateral lips and the oesophagus was divided into 2 distinct regions (described above). These morphological features were consistent with those of other spiruroid larvae.

Prevalence and distribution:

Infections were only detected in a total of 36 yabbies; 31 originating from wild populations and 5 from farmed populations. The levels of infection were generally mild with only several nodules being detected in individual yabbies. No infections were detected in yabbies from the mid-North whereas the prevalence of infections ranged from 1-2% in the other regions sampled throughout the state.

Significance of infections by spiruroid larvae

The nodules containing the spiruroid larvae were not apparently associated with any clinical disease or pathological condition. No previous studies have reported the detection of similar parasites in yabbies. The yabbies presumably function as intermediate hosts for these larval stages which must be ingested by a definitive host for the development of adult worms. The yabbies probably become infected following the ingestion of worm eggs shed in the faeces of the definitive host. It is not known which mammal or bird species serves as the definitive host for these parasites. Nevertheless, infections may be controlled to some extent by restricting predation on yabbies by preventing the access of predatory or scavenging animals and birds to ponds.

Although infections were only detected in yabbies and not in marron, it is not known whether the parasites were strictly host specific. Infections were not very prevalent throughout the state and they are not thought to be present any serious problem to crayfish aquaculture.

IV. ACANTHOCEPHALA (Kingdom Animalia: Phylum Acanthocephala)

Infections by developmental stages of acanthocephalan worms (thorny-headed worms) were detected in the gut walls of crayfish. Acanthocephalans were formerly grouped with the cestodes but they are now recognized as a separate phylum due to their different mode of nutrition (use pores and canals in cuticle instead of absorption through general body surface). They are exclusively intestinal parasites of vertebrates which have larval developmental stages in invertebrates.

A. Thorny-headed worms (Class Acanthocephala: Order Palaeacanthocephala)

The developmental stages detected in the crayfish were the larval forms (cystacanths) of a single acanthocephalan species. The cystacanths were relatively small in size and contained a single larval stage with an inverted proboscis.

Polymorphus biziurae (Order Palaeacanthocephala: Family Polymorphidae)

Morphological characteristics (see Figures 43 and 44):

Small pink ovoid cysts around 2 mm in diameter were detected attached to the intestinal mucosa of the crayfish. When the cysts were removed and placed in tap water, a proboscis extended or everted from the body of each larva. The everted proboscis was 0.5-1.0 mm long and armed with numerous spines. The spines were arranged in 16 longitudinal rows each containing 7-10 hooks. The species was identified as P. biziurae which is a common parasite of musk ducks.

Prevalence and distribution:

Infections were only detected in a total of 7 yabbies which all originated from wild populations. The majority of infections involved single cystacanth stages in individual yabbies. Infections were detected in 4 yabbies from Kangaroo Island, 2 from the South-East and one from the River Murray.

Significance of acanthocephalan infections

The acanthocephalan infections were detected as larval cystacanth stages in the gut tissues of the yabbies which served as intermediate hosts for the parasites. The cystacanths must be ingested by the definitive host species (musk ducks) for the development of the adult acanthocephalan. The yabbies probably become infected following the ingestion of acanthocephalan eggs which are passed in the faeces of the definitive host. The eggs usually resemble spindle-shaped diatoms which makes them a favourable food for crustacea. Infections may be controlled by interrupting the parasite life cycle by preventing the access of predatory waterbirds to yabbie ponds.

The cystacanth stages were not directly associated with any clinical disease or pathological condition in the yabbies. Infections were very low in prevalence and were restricted in their distribution around the state. They are therefore not regarded as being of significance to the crayfish aquaculture industry.

SUMMARY

A total of 17 invertebrate genera were detected in association with the freshwater crayfish examined in this study; 6 genera being detected as endoparasites within crayfish tissues and another 11 genera being found as ectocommensal or epizoic organisms on crayfish.

Parasites

Infections by 3 genera of parasites do represent causes of concern to the aquaculture industry due to various combinations of factors; including their pathogenic potential, their deleterious effects on host musculature and their distribution and abundance within South Australia. The microsporidian parasites Thelohania and Pleistophora and the trematode parasite Microphallus all form cyst-like structures within the muscles of crayfish. Heavy infections can affect the appearance of the muscles making them unsuitable for marketing. Infections by Thelohania have also previously been associated with crayfish mortalities interstate.

In the present study, infections by these 3 parasites were detected almost exclusively in yabbies which had been sampled from wild populations. Although the distribution and abundance of the parasites varied throughout the state, certain regions were identified where infections were most prevalent; namely, the Broughton River and the River Light in the mid-North of the state. It is therefore recommended that yabbies from these river systems not be used to stock culture ponds anywhere in Australia due to the high levels of infections. Furthermore, any enterprises established in the mid-North of South Australia should take all precautions to prevent the introduction or migration of wild yabbies into their culture facilities. It is further recommended that imported crayfish species be examined for infections by these parasites to avoid their introduction from other areas.

The remainder of the parasites (Vampirolepis, Polymorphus and the spiruroid larvae) were not associated with any clinical diseases or pathological conditions and are therefore not regarded as significant pathogens of crayfish.

Ectocommensals

Infestations by temnocephalan platyhelminths do present a different kind of problem for the aquaculture industry. Temnocephalan eggs attached to the exoskeletons of crayfish are strongly adherent and remain in place following cooking. Their presence on crayfish makes them unsightly and may lead to consumer resistance. In addition, previous studies have found that severe infestations within the gill chambers may cause hypoxia and even death. At present, few suitable measures are available for the prevention or control of infestations. Their high prevalence and wide distribution throughout the state may also limit any attempts to establish farm populations free of infestations.

The remainder of the ectocommensal organisms (ciliates and free-living nematodes) are regarded as fouling organisms which are not pathogenic by themselves. However, there is mounting evidence that severe infestations of the gills may affect respiratory efficiency, cause hypoxia and even death. Heavy infestations are usually indicative of poor water quality particularly involving high nutrient levels. Fortunately, various strategies are available to improve water quality which subsequently alleviates infestations by fouling organisms.

CONCLUSIONS

It is recommended that movement restrictions be imposed on freshwater crayfish between interstate and intrastate watersheds subject to health certification testing for infections by microsporidian cysts (Thelohania and Pleistophora). In addition, extension programmes should be devised to encourage producers to routinely monitor crayfish stock for infections by these microsporidian parasites as well as those by trematode metacercariae (Microphallus).

Figure 3. Vaginicola (Protozoa: Ciliophora: Peritrichida). Peritrichous ciliates found in gill cavities of yabbies and marron. (Phase-contrast micrograph).

Figure 4. Vaginicola (Protozoa: Ciliophora: Peritrichida). Upright loricae attached to gill filament. (Scanning electron micrograph).

Figure 5. Cothurnia (Protozoa: Ciliophora: Peritrichida). Peritrichous ciliates detected in gill cavities of yabbies and marron. (Scanning electron micrograph).

Figure 6. Cothurnia (Protozoa: Ciliophora: Peritrichida). Upright stalked loricae attached to gill filament. (Scanning electron micrograph).

Figure 7. Pyxicola (Protozoa: Ciliophora: Peritrichida). Peritrichous ciliates bearing discoid operculum contained within upright stalked lorica on pleopod of yabbies. (Phase-contrast micrograph).

Figure 8. Lagenophrys (Protozoa: Ciliophora: Peritrichida). Peritrichous ciliate found on gills of yabbies and marron. (Phase-contrast micrograph).

Figure 9. Lagenophrys (Protozoa: Ciliophora: Peritrichida). Numerous loricae covering gill filament. (Scanning electron micrograph).

Figure 10. Lagenophrys (Protozoa: Ciliophora: Peritrichida). Flat loricae attached to gill filament. (Scanning electron micrograph).


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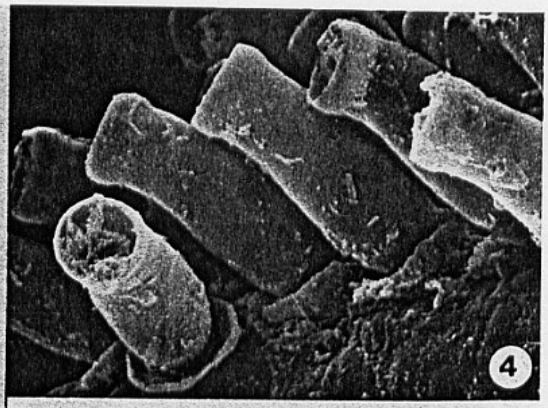
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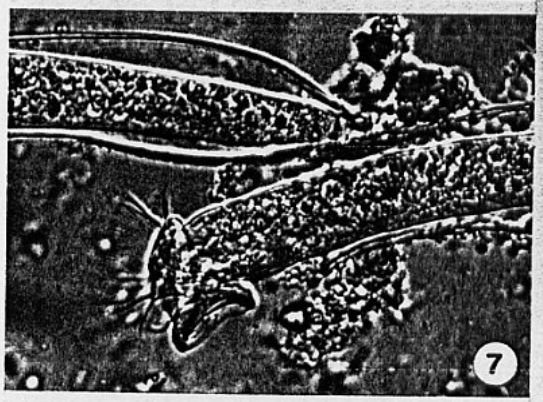
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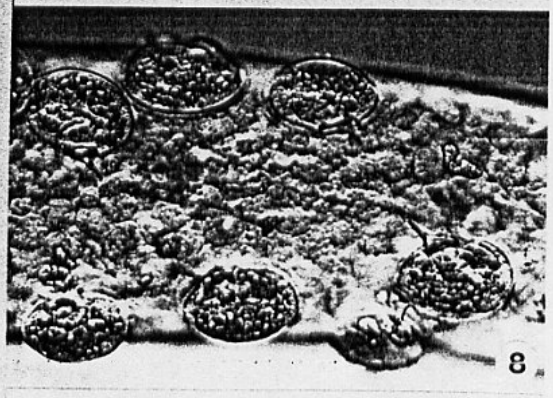
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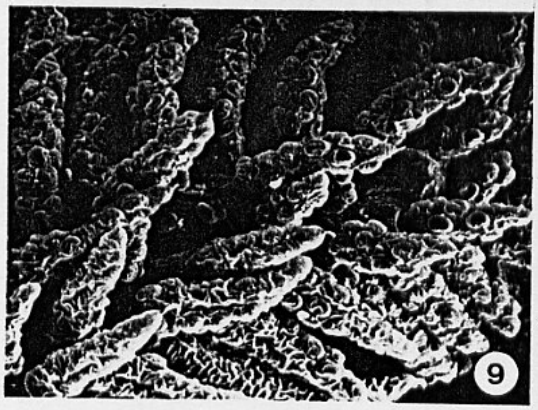
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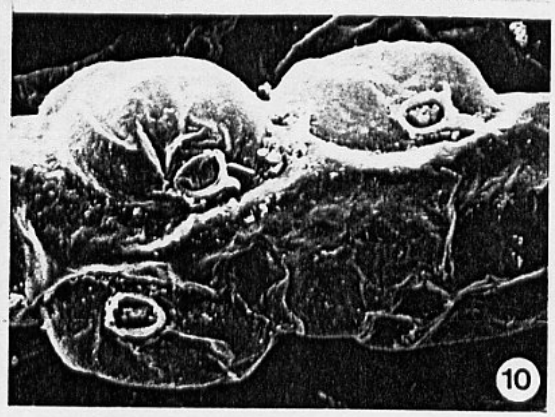
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Figure 11. Epistylis (Protozoa: Ciliophora: Peritrichida). Peritrichous ciliates on branched non-contractile stalks attached to exoskeletons of yabbies and marron. (Phase-contrast micrograph).

Figure 12. Epistylis (Protozoa: Ciliophora: Peritrichida). Contracted zooids attached to pleopod. (Scanning electron micrograph).

Figure 13. Epistylis (Protozoa: Ciliophora: Peritrichida). Ridged pellicle of contracted zooid. (Scanning electron micrograph).

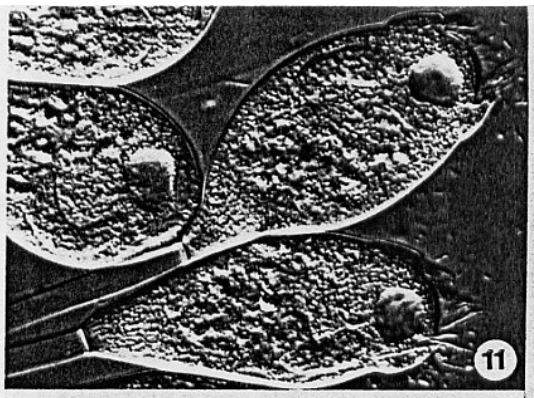
Figure 14. Vorticella (Protozoa: Ciliophora: Peritrichida). Peritrichous ciliates on non-branched contractile stalks attached to exoskeleton of yabbies and marron. (Phase-contrast micrograph).

Figure 15. Vorticella (Protozoa: Ciliophora: Peritrichida). Contracted zooids on stalks attached to pleopod. (Scanning electron micrograph).

Figure 16. Acineta (Protozoa: Ciliophora: Suctorida). Suctorian ciliate in loricae attached to pleopods of yabbies and marron. (Phase-contrast micrograph).

Figure 17. Acineta (Protozoa: Ciliophora: Suctorida). Zooid with two fascicles of tentacles. (Scanning electron micrograph).

Figure 18. Tokophrya (Protozoa: Ciliophora: Suctorida). Suctorian ciliate attached to pleopod of yabbie. (Phase-contrast micrograph).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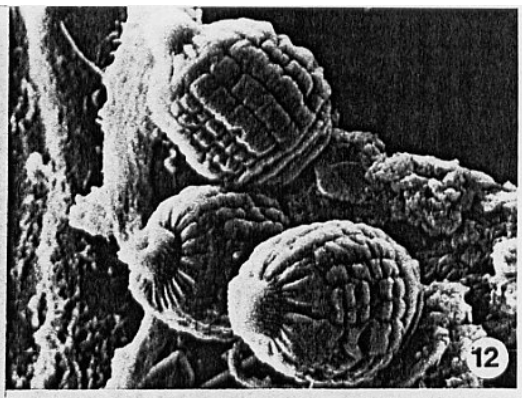
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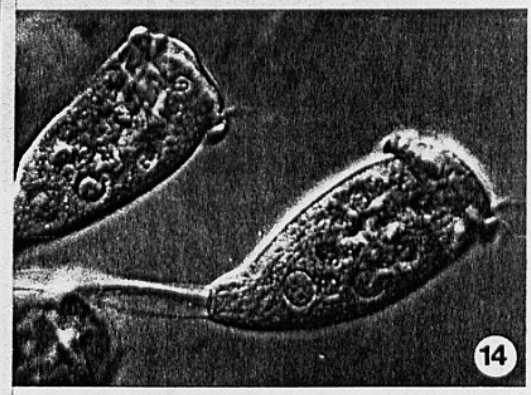
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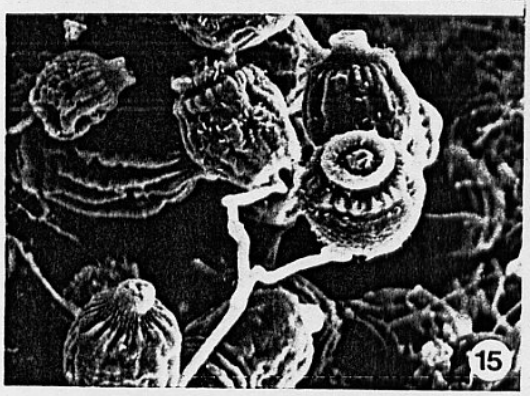
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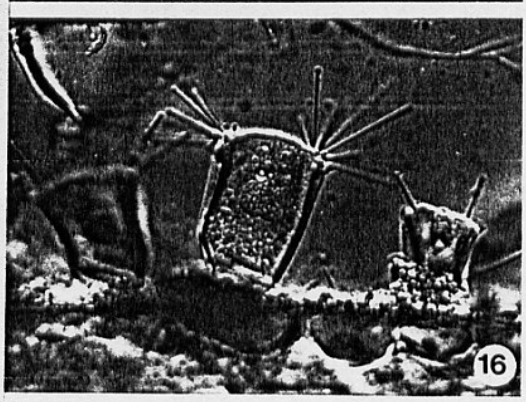
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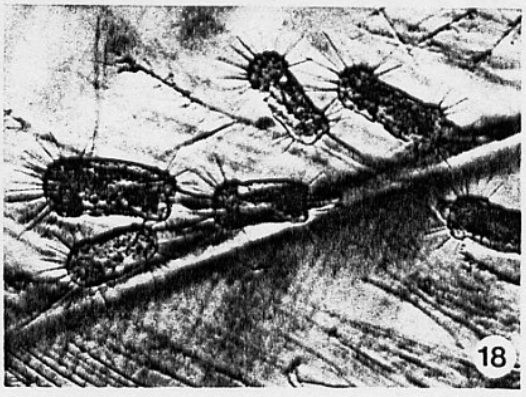
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Figure 19. Thelohania (Protozoa: Microspora: Pleistophorida).
Cross-section through yabbie muscle fibres packed with microsporidian cysts. (Light micrograph, Ziehl-Neelsen stain).

Figure 20. Thelohania (Protozoa: Microspora: Pleistophorida).
Longitudinal section through cyst packed with mature spores and sporoblasts. (Light micrograph, Giemsa stain).

Figure 21. Thelohania (Protozoa: Microspora: Pleistophorida).
Sporophorous vesicle containing 8 sporoblasts amongst numerous free mature spores.
(Nomarski differential interference contrast micrograph).

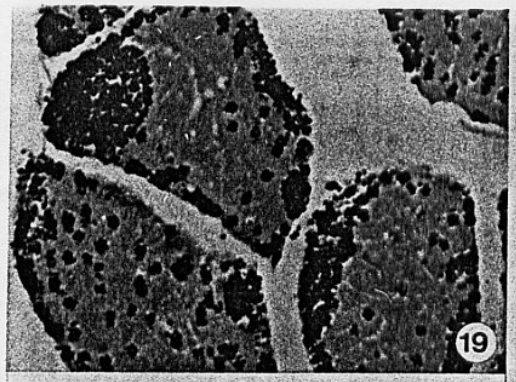
Figure 22. Pleistophora (Protozoa: Microspora: Pleistophorida).
Cross-section through large microsporidian cyst bounded by thick wall. (Light micrograph, Giemsa stain).

Figure 23. Pleistophora (Protozoa: Microspora: Pleistophorida).
Section through periphery of cyst packed with spores showing invagination of cyst wall.
(Light micrograph, haematoxylin and eosin stain).

Figure 24. Pleistophora (Protozoa: Microspora: Pleistophorida).
Squash preparation of cyst packed with mature spores.
(Nomarski differential interference contrast micrograph).

Figure 25. Pleistophora (Protozoa: Microspora: Pleistophorida).
Squash preparation of cyst containing sporophorous vesicles packed with irregular numbers of spores.
(Nomarski differential intereference contrast micrograph).

Figure 26. Pleistophora (Protozoa: Microspora: Pleistophorida).
Squash preparation of sporophorous vesicles filled with numerous micro-spores.
(Nomarski differential interference contrast micrograph).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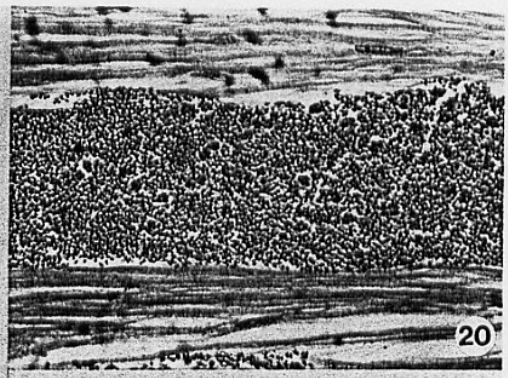
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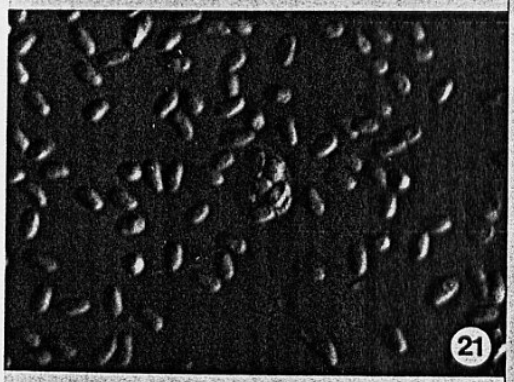
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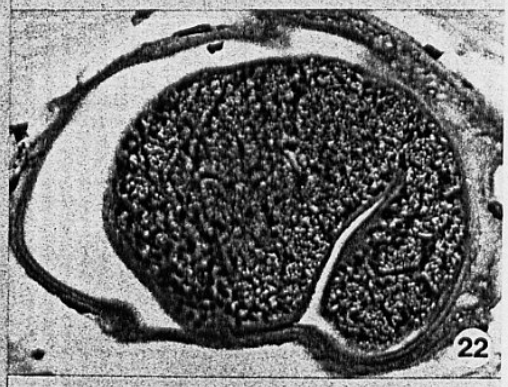
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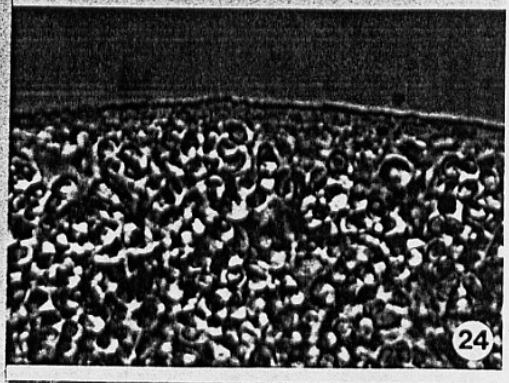
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Figure 27. Microphallus minutus (Platyhelminthes: Trematoda: Digenea). Metacercaria of digenean fluke in tail muscles of wild yabbies. (Light micrograph).

Figure 28. Microphallus minutus (Platyhelminthes: Trematoda: Digenea). Sections through metacercariae in tail musculature. (Light micrograph, haematoxylin and eosin stain).

Figure 29. Microphallus (Platyhelminthes: Trematoda: Digenea) and Pleistophora (Protozoa: Microspora: Pleistophorida). Section through metacercaria on left packed with numerous microsporidian spores (presumably Pleistophora). (Light micrograph, Ziehl-Neelsen stain).

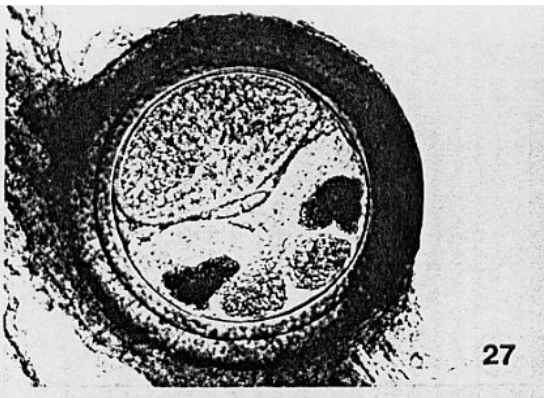
Figure 30. Microphallus (Platyhelminthes: Digenea: Microphallidea) and Pleistophora (Protozoa: Microspora: Pleistophorida). Remnants of sucker in trematode metacercarial stage packed with mature spores. (Light micrograph, Giemsa stain).

Figure 31. Craspedella spenceri (Platyhelminthes: Temnocephala: Temnocephalidea Flatworm with three transverse lamellae (or frills) found browsing over exoskeleton of both yabbies and marron. (Light micrograph).

Figure 32. Temnocephala dendyi (Platyhelminthes: Temnocephala: Temnocephalidea) Unpigmented temnocephalid found on yabbies and marron. (Light micrograph).

Figure 33. Temnocephala sp. (Platyhelminthes: Temnocephala: Temnocephalidea). Undescribed pigmented temnocephalid found on exoskeleton of yabbies. (Light micrograph).

Figure 34. Temnocephala sp. (Platyhelminthes: Temnocephala: Temnocephalidea). Tentacles and eyespots of undescribed pigmented temnocephalid from yabbies. (Light micrograph).



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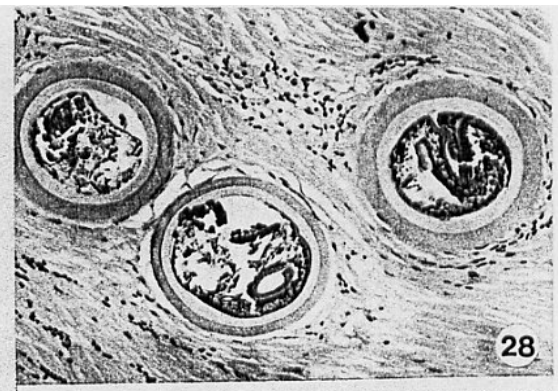

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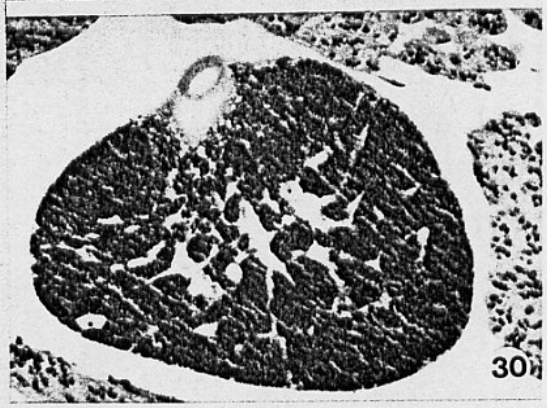

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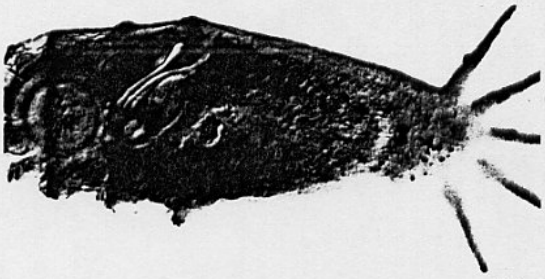

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Figure 35. Vampirolepis (Platyhelminthes: Cestoda: Cyclophyllidea). Cysticercoid larval stage of cestode (tapeworm) from gut of wild yabbie. (Light micrograph).

Figure 36. Vampirolepis (Platyhelminthes: Cestoda: Cyclophyllidea). Encysted scolex (head) of cestode larva from gut of yabbie. (Light micrograph).

Figure 37. Gammarinema (Nematoda: Chromadorea: Monhysterida). Adult nematode (roundworm) from gill cavity of yabbie. (Light micrograph).

Figure 38. Gammarinema (Nematoda: Chromadorea: Monhysterida). Head of adult nematode from gill cavity. (Nomarski differential interference contrast micrograph).

Figure 39. Gammarinema (Nematoda: Chromadorea: Monhysterida). Tail of adult male with projecting spicule. (Nomarski differential interference contrast micrograph).

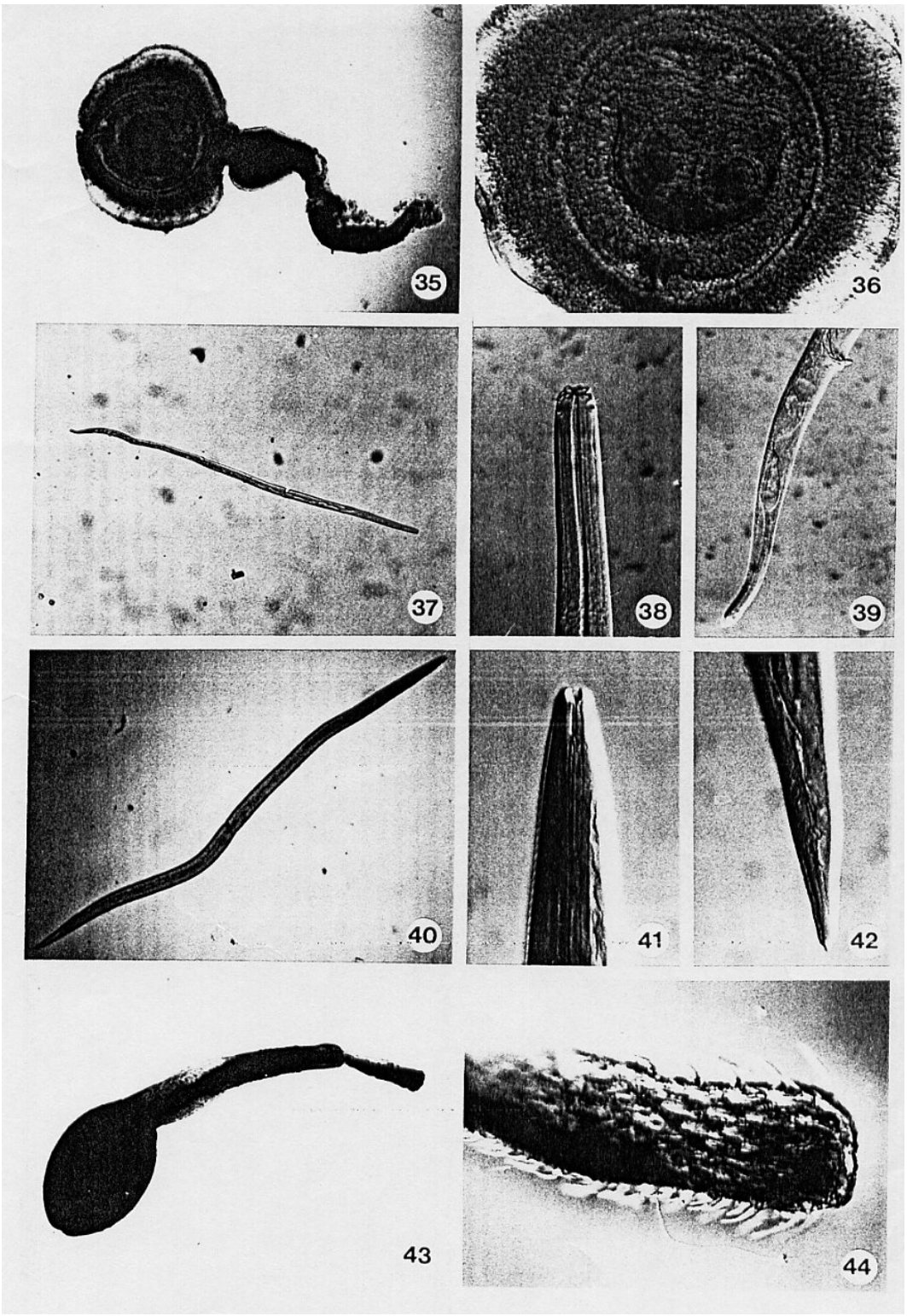
Figure 40. Spiruroid larvae (Nematoda: Rhabditea: Spirurida). Nematode (roundworm) larva from gut of yabbie. (Light micrograph).

Figure 41. Spiruroid larvae (Nematoda: Rhabditea: Spirurida). Head of nematode larva from gut of yabbie. (Nomarski differential interference contrast micrograph).

Figure 42. Spiruroid larvae (Nematoda: Rhabditea: Spirurida). Tail of nematode larva from gut of yabbie. (Nomarski differential interference contrast micrograph).

Figure 43. Polymorphus (Acanthocephala: Palaeacanthocephala: Polymorphidae). Cystacanth larval stage of acanthocephalan (thorny-headed worm) from gut of yabbie. (Light micrograph).

Figure 44. Polymorphus (Acanthocephala: Palaeacanthocephala: Polymorphidae). Everted proboscis armed with hooks. (Light micrograph).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